Calculations of double spike isotope dilution results revisited†

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The use of isotope pattern deconvolution analysis is proposed to simplify the double spiking species-specific isotope dilution result calculations when inter-conversion might occur. A detailed example is given for the determination of Cr(III)/Cr(VI) in yeast by isotope dilution-HPLC-ICP-MS. The results are in exact agreeent with the conventional isotope dilution calculations.

Introduction

Isotope dilution (ID) mass spectrometry is one of the most valuable tools in modern analytical chemistry.[1] The main reason for this is that the analytical result is not biased by matrix effects or losses of analyte if proper analysis protocols are followed. As an extension of the classical ID approach, multiple spiking offers many advantages, most importantly, perhaps, accurate quantitation in the presence of species inter-conversion during sample preparation. In 1997 Hintelman *et al.* pointed out that published CH_3Hg^+ concentrations should be viewed with caution due to the formation of artificial methylmercury from the added inorganic Hg(II) spike.[2] The resulting overestimation of methylmercury in sediments was reported to be as high as 80%. Although a simple standard addition procedure can compensate for the artificial CH_3Hg^+ formation,[2] double spiking ID can provide the species inter-conversion corrections in a single measurement.[3]

Even though advanced ID methods give results that are immune to the species degradation and inter-conversion, one of the main obstacles for actual dissemination of such methods (*e.g.*, multiple spiking) is the cumbersome calculations that need to be performed to obtain the analytical results. In this report we show that the matrix notation based deconvolution approach to isotope dilution greatly simplifies the data analysis from the multiple spike experiments.

The analysis of aqueous Cr(III) and Cr(VI) is a classical example where bidirectional inter-conversion occurs.[4,5] Consider the Cr(III) and Cr(VI) determination in aqueous solutions using ion-exchange HPLC-ICP-MS with two added spikes: (1) ^{50}Cr-enriched Cr(III) and (2) ^{53}Cr-enriched Cr(VI). In this method three Cr isotopes are monitored: ^{50}Cr, ^{52}Cr and ^{53}Cr. Accordingly, we have five different isotopic signatures: the natural isotope pattern of both analytes—Cr(III) and Cr(VI); two distinct isotope patterns of enriched spikes—^{50}Cr(III) and ^{53}Cr(VI); and the two measured isotope patterns in both chromatographic peaks of Cr(III) and Cr(VI).

It is known that bidirectional inter-conversion of Cr(III) and Cr(VI) can occur during the analysis and this process can be described in the following scheme:

$$\mathrm{Cr(III)} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \mathrm{Cr(VI)}$$

According to this notation, α represents the relative amount of any Cr(III) species (enriched or natural) oxidized to Cr(VI) during the sample preparation and β represents the relative amount of Cr(VI) species reduced to Cr(III). Thus, the analyst has four unknown variables: α, β, and the concentrations of Cr(III) and Cr(VI) in the sample. Conventionally, these four unknown variables are calculated from the system of four equations each representing the m/z 50/52 and 53/52 isotopic ratios in both chromatographic peaks of Cr(III) and Cr(VI):[4–6]

$$\begin{cases} \dfrac{I^{50}_{\mathrm{Cr(III)}}}{I^{52}_{\mathrm{Cr(III)}}} = \dfrac{\left(A^{50}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s50}_{\mathrm{Cr(III)}}n^{s}_{\mathrm{Cr(III)}}\right)(1-\alpha)+\left(A^{50}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s50}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)\beta}{\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s52}_{\mathrm{Cr(III)}}n^{s}_{\mathrm{Cr(III)}}\right)(1-\alpha)+\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s52}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)\beta} \\ \dfrac{I^{53}_{\mathrm{Cr(III)}}}{I^{52}_{\mathrm{Cr(III)}}} = \dfrac{\left(A^{53}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s53}_{\mathrm{Cr(III)}}n^{s}_{\mathrm{Cr(III)}}\right)(1-\alpha)+\left(A^{53}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s53}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)\beta}{\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s52}_{\mathrm{Cr(III)}}n^{s}_{\mathrm{Cr(III)}}\right)(1-\alpha)+\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s52}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)\beta} \\ \dfrac{I^{50}_{\mathrm{Cr(VI)}}}{I^{52}_{\mathrm{Cr(VI)}}} = \dfrac{\left(A^{50}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s50}_{\mathrm{Cr(III)}}n^{s}_{\mathrm{Cr(III)}}\right)\alpha+\left(A^{50}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s50}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)(1-\beta)}{\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s52}_{\mathrm{Cr(III)}}n^{s}_{\mathrm{Cr(III)}}\right)\alpha+\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s52}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)(1-\beta)} \\ \dfrac{I^{53}_{\mathrm{Cr(VI)}}}{I^{52}_{\mathrm{Cr(VI)}}} = \dfrac{\left(A^{53}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s53}_{\mathrm{Cr(III)}}n^{s}_{\mathrm{Cr(III)}}\right)\alpha+\left(A^{53}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s53}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)(1-\beta)}{\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(III)}}+A^{s52}_{\mathrm{Cr}}n^{s}_{\mathrm{Cr(III)}}\right)\alpha+\left(A^{52}_{\mathrm{Cr}}n_{\mathrm{Cr(VI)}}+A^{s52}_{\mathrm{Cr(VI)}}n^{s}_{\mathrm{Cr(VI)}}\right)(1-\beta)} \end{cases}$$

where I stands for the mass-bias and background corrected intensities of Cr(III) and Cr(VI). A_{Cr} are the natural isotopic abundances of chromium, $A^{s}_{\mathrm{Cr(III)}}$ and $A^{s}_{\mathrm{Cr(VI)}}$ are those of the Cr(III) and Cr(VI) spikes and n is the amount of the corresponding species. Superscripts denote the mass of the isotope and "*s*" stands for "spike". Even though each of these four equations contains about 20 mathematical operations (summation and multiplication) and can be manipulated by analytical chemists, mathematical expression of β from these equations yields an expression with about 270 operations—a complexity clearly manageable only by computer. As a result of such complexity the above equations are prone to human errors. As well, the size of the above conventional two-isotope ratio based equations prohibits their circulation in presentations and published literature, thus leaving the mathematical aspects of multiple spike ID to be one of the least attractive and eventually the least accessible areas of the field. Owing to

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† Electronic supplementary information (ESI) available: Microsoft® Excel spreadsheet for calculating Cr(III) and Cr(VI) concentrations from the double spiking experiments. See DOI: 10.1039/b607823k.

the complexity of these four expressions, USEPA recommends solving the above system of equations iteratively.[6] Usually, about 6–8 iterations are needed to obtain all four unknowns. An alternative to solving highly complex systems of equations (such as this example) is using commercial numerical calculation software such as Matlab or Mathcad: however, such software packages are not always available in chemical laboratories.

We propose to simplify the double spike isotope dilution calculations with species interconversion by abandoning the conventional two-isotope ratio approach. Alternatively, isotope dilution results can be obtained using a regression based isotope pattern deconvolution which simplifies the working expressions thus eliminating the need for iterative calculations.

Isotope pattern deconvolution

Consider the analyte and the isotopically enriched spike with isotope patterns spanning n m/z values. Their isotopic compositions are $(A_a^1,A_a^2,\ldots,A_a^n)$ and $(A_s^1,A_s^2,\ldots,A_s^n)$, respectively. In most of the cases some of the minor isotopes are not monitored, therefore usually $\Sigma A_a,\Sigma A_s < 1$. When N_a molecules/atoms of the analyte (a) are mixed with N_s molecules/atoms of the spike (s), the measured isotopic intensities of the mixture (m) are $(I_m^1,I_m^2,\ldots,I_m^n)$. Since $N_m = N_a + N_s$, the following is true for the mixture:

$$\begin{pmatrix} N_a^1 \\ N_a^2 \\ \ldots \\ N_a^n \end{pmatrix} + \begin{pmatrix} N_s^1 \\ N_s^2 \\ \ldots \\ N_s^n \end{pmatrix} = \begin{pmatrix} N_m^1 \\ N_m^2 \\ \ldots \\ N_m^n \end{pmatrix} \quad (1)$$

This equation can be rewritten using the isotopic abundances:

$$N_a \cdot \begin{pmatrix} A_a^1 \\ A_a^2 \\ \ldots \\ A_a^n \end{pmatrix} + N_s \cdot \begin{pmatrix} A_s^1 \\ A_s^2 \\ \ldots \\ A_s^n \end{pmatrix} = N_m \cdot \begin{pmatrix} A_m^1 \\ A_m^2 \\ \ldots \\ A_m^n \end{pmatrix} \quad (2)$$

$$N_a \cdot \begin{pmatrix} A_a^1 \\ A_a^2 \\ \ldots \\ A_a^n \end{pmatrix} + N_s \cdot \begin{pmatrix} A_s^1 \\ A_s^2 \\ \ldots \\ A_s^n \end{pmatrix} = (N_a + N_s)s \cdot \begin{pmatrix} I_m^1 \\ I_m^2 \\ \ldots \\ I_m^n \end{pmatrix} \quad (3)$$

Here s stands for the (unknown) instrumental sensitivity coefficient that relates isotopic abundances with the measured isotopic intensities. Eqn (3) can be further simplified:

$$\underbrace{\frac{N_a}{(N_a + N_s)s}}_{b_1} \cdot \begin{pmatrix} A_a^1 \\ A_a^2 \\ \ldots \\ A_a^n \end{pmatrix} + \underbrace{\frac{N_s}{(N_a + N_s)s}}_{b_2} \cdot \begin{pmatrix} A_s^1 \\ A_s^2 \\ \ldots \\ A_s^n \end{pmatrix} = \begin{pmatrix} I_m^1 \\ I_m^2 \\ \ldots \\ I_m^n \end{pmatrix} \quad (4)$$

In a matrix notation eqn (4) is identical to the following expression:

$$\begin{pmatrix} I_m^1 \\ I_m^2 \\ \ldots \\ I_m^n \end{pmatrix} = \begin{pmatrix} A_a^1 & A_s^1 \\ A_a^2 & A_s^2 \\ \ldots & \ldots \\ A_a^n & A_s^n \end{pmatrix} \cdot \begin{pmatrix} b_1 \\ b_2 \end{pmatrix} \quad (5)$$

The equation obtained can be interpreted as multiple linear regression with two slopes—b_1 and b_2. The maximum likelihood estimates of these slopes can be obtained using a standard least-squares algorithm.[7,8] The ratio of these slopes defines the relationship between the amount of analyte and spike atoms/molecules (from eqn (4)):

$$\frac{b_1}{b_2} = \frac{N_a}{N_s} = \frac{n_a}{n_s} \quad (6)$$

Here N is the number of molecules/atoms of the analyte and spike in the aliquots and n is their corresponding amount (mol). Since the amount of added spike (n_s) is known, eqn (6) contains a single unknown—the amount of analyte in the sample (n_a).

This equation is the central theme of the isotope dilution analysis using isotope pattern deconvolution and it demonstrates the simple relationship between the amounts of involved substances (analyte and spike) and the regression slopes. Applications of this equation will be demonstrated in the next sections. Note that the proposed isotope pattern dilution analysis is, in principle, the reverse problem of isotope pattern reconstruction.

Application to Cr(III)/Cr(VI) speciation

The experimental results of the Cr(III) and Cr(VI) quantitation experiment can be arranged in three matrices. The first two matrices are the measured m/z 50, 52 and 53 ion intensities in both chromatographic peaks of Cr(III) and Cr(VI) (left sides of the eqns (7) and (8)). The third matrix (right sides of the eqns (7) and (8)) consists of three stacked columns, each representing the isotopic abundances of the Cr(III) spike (left), the Cr(VI) spike (middle), and natural Cr (right):

$$\begin{matrix} 50 \\ 52 \\ 53 \end{matrix} \begin{pmatrix} I_{Cr(III)} \\ \ldots \\ I_{Cr(III)} \end{pmatrix} = \begin{pmatrix} A^s_{Cr(III)} & A^s_{Cr(VI)} & A_{Cr} \\ \ldots & \ldots & \ldots \\ A^s_{Cr(III)} & A^s_{Cr(VI)} & A_{Cr} \end{pmatrix} \cdot \begin{pmatrix} a_1 \\ b_1 \\ c_1 \end{pmatrix} \quad (7)$$

$$\begin{matrix} 50 \\ 52 \\ 53 \end{matrix} \begin{pmatrix} I_{Cr(VI)} \\ \ldots \\ I_{Cr(VI)} \end{pmatrix} = \begin{pmatrix} A^s_{Cr(III)} & A^s_{Cr(VI)} & A_{Cr} \\ \ldots & \ldots & \ldots \\ A^s_{Cr(III)} & A^s_{Cr(VI)} & A_{Cr} \end{pmatrix} \cdot \begin{pmatrix} a_2 \\ b_2 \\ c_2 \end{pmatrix} \quad (8)$$

The measured (mass-bias corrected) peak areas of ions m/z 50, 52 and 53 in Cr(III) and Cr(VI) peaks are related to the natural and spike Cr abundance matrices *via* the two coefficient matrices (a_1, b_1, c_1) and (a_2, b_2, c_2). The latter are essentially multiple regression coefficient matrices and can be obtained using the LINEST array function in Microsoft® Excel (by setting the regression intercept to be zero). The physical meaning of the matrices (a_1, b_1, c_1) and (a_2, b_2, c_2) is that they provide information about how much each of the columns from the right side of eqns (7) and (8) contribute to the measured isotope intensities in Cr(III) and Cr(VI) peaks. In other words, coefficient a_1 is proportional to the amount of Cr(III) spike contributing to the Cr(III) in the measured chromatographic peak. The values of the Cr(III) and Cr(VI) interconversion parameters α and β are directly linked with the slopes a, b, and c, as shown in Scheme 1. For example, the slope b_1 is proportional to the flux of Cr(VI) spike reduction into Cr(III). If no reduction from Cr(VI) to Cr(III) occurs, then $b_1 = 0$. Essentially, the obtained regression slopes are proportional to the amounts of each species in the sample at the time of analysis. The ratios of these slopes within each set (a_1, b_1, c_1) and (a_2, b_2, c_2) can be directly used to obtain the values of α

$$
\begin{array}{lll}
 & \leftarrow a_1 - & (1-\alpha)\cdot n_{\mathrm{Cr(III)}} \\
\mathrm{Cr(III)} & \leftarrow b_1 - & \beta\cdot n^{s}_{\mathrm{Cr(VI)}} \\
 & \leftarrow c_1 - & (1-\alpha)\cdot n_{\mathrm{Cr(III)}} + \beta\cdot n_{\mathrm{Cr(VI)}} \\
\hline
 & \leftarrow a_2 - & \alpha\cdot n^{s}_{\mathrm{Cr(III)}} \\
\mathrm{Cr(VI)} & \leftarrow b_2 - & (1-\beta)\cdot n^{s}_{\mathrm{Cr(VI)}} \\
 & \leftarrow c_2 - & \alpha\cdot n_{\mathrm{Cr(III)}} + (1-\beta)\cdot n_{\mathrm{Cr(VI)}}
\end{array}
$$

Scheme 1 Relationship between the multiple regression slopes, species inter-conversion coefficients α and β and their amounts in the sample.

and β (refer to Scheme 1):

$$\frac{a_1}{b_1} = \frac{(1-\alpha)\cdot n^{enr}_{\mathrm{Cr(III)}}}{\beta\cdot n^{enr}_{\mathrm{Cr(VI)}}}, \text{ and } \frac{a_2}{b_2} = \frac{\alpha\cdot n^{enr}_{\mathrm{Cr(III)}}}{(1-\beta)\cdot n^{enr}_{\mathrm{Cr(VI)}}} \qquad (9)$$

From here the values of α and β can be expressed as follows:

$$\alpha = \frac{a_1 a_2}{a_1 b_2 - a_2 b_1}\cdot\left(\frac{n^{s}_{\mathrm{Cr(VI)}}}{n^{s}_{\mathrm{Cr(III)}}} - \frac{b_1}{a_1}\right), \text{ and}$$
$$\beta = (1-\alpha)\frac{b_1}{a_1}\cdot\frac{n^{s}_{\mathrm{Cr(VI)}}}{n^{s}_{\mathrm{Cr(III)}}} \qquad (10)$$

Once the inter-conversion coefficients α and β are obtained, the two remaining unknowns are the amounts of Cr(III) and Cr(VI) in the analysed sample. These can be calculated similar to α and β, this time using the ratio of the slopes c_1, a_1 and c_2, b_2 (see Scheme 1):

$$\begin{cases} (1-\alpha)\cdot n_{\mathrm{Cr(III)}} + \beta\cdot n_{\mathrm{Cr(VI)}} = \dfrac{c_1}{a_1}(1-\alpha)\cdot n^{enr}_{\mathrm{Cr(III)}} = A(1-\alpha) \\ \alpha\cdot n_{\mathrm{Cr(III)}} + (1-\beta)\cdot n_{\mathrm{Cr(VI)}} = \dfrac{c_2}{b_2}(1-\beta)\cdot n^{enr}_{\mathrm{Cr(VI)}} = B(1-\beta) \end{cases} \qquad (11)$$

The amounts of Cr(III) and Cr(VI) can be obtained, for example, using the Kramer's matrix determinant approach:

$$n_{\mathrm{Cr(III)}} = \frac{\begin{vmatrix} A(1-\alpha) & \beta \\ B(1-\beta) & 1-\beta \end{vmatrix}}{\begin{vmatrix} 1-\alpha & \beta \\ \alpha & 1-\beta \end{vmatrix}}, \text{ and}$$
$$n_{\mathrm{Cr(VI)}} = \frac{\begin{vmatrix} 1-\alpha & A(1-\alpha) \\ \alpha & B(1-\beta) \end{vmatrix}}{\begin{vmatrix} 1-\alpha & \beta \\ \alpha & 1-\beta \end{vmatrix}} \qquad (12)$$

Solution of these equations yields the analyte amounts in the sample, $n_{\mathrm{Cr(III)}}$ and $n_{\mathrm{Cr(VI)}}$:

$$n_{\mathrm{Cr(III)}} = \frac{A(1-\alpha)(1-\beta) - B\beta(1-\beta)}{1-\alpha-\beta}$$
$$n_{\mathrm{Cr(VI)}} = \frac{B(1-\alpha)(1-\beta) - A\alpha(1-\alpha)}{1-\alpha-\beta} \qquad (13)$$

In a special situation, when no species inter-conversion occurs, *i.e.*, $\alpha = \beta = 0$, the above expressions reduce to $n_{\mathrm{Cr(III)}} = A$ and $n_{\mathrm{Cr(VI)}} = B$. Also, note that all of the above expressions are in exact agreement with the conventional two-isotope ratio calculations adopted world-wide.[4–6] Moreover, the matrix-based approach reduces the computational complexity, thus avoiding the need for a time-consuming iterative approach.

Example calculation

As an illustrative example of the above equations, Cr(III) and Cr(VI) determination in yeast by HPLC-ICP-MS will be used to demonstrate the principle of the least-squares ID approach.[9] Yeast samples were digested at 95 °C in alkaline solution and the resulting solution was analysed using anion-exchange liquid chromatography. 0.2025 g of the sample was spiked with 1.0145 g of ^{50}Cr(III) spike (7.3115 μmol Cr g^{-1} as obtained from the reverse ID) and 1.0078 g of ^{53}Cr(VI) spike (13.5357 μmol Cr g^{-1}). The isotopic abundances of the chromium species used were as follows:

	^{50}Cr(III)	^{53}Cr(VI)	Cr(nat)
50	0.9720	0.0004	0.0435
52	0.0258	0.0284	0.8379
53	0.0019	0.9698	0.0950

Isotope abundances of spikes are usually known directly from the analysis certificates and those of the natural Cr can be obtained from IUPAC.[10] Note that the sum of m/z 50, 52, and 53 ion abundances does not add to one ($\Sigma A_{\mathrm{Cr(III)}} = 0.9997$, $\Sigma A_{\mathrm{Cr(VI)}} = 0.9986$ and $\Sigma A_{\mathrm{Cr}} = 0.9764$) due to the presence of the ^{54}Cr isotope not being monitored in this case.

The m/z 50, 52 and 53 ion peak areas were measured from both chromatographic peaks of Cr(III) and Cr(VI) (see Fig. 1), and the raw peak areas were then background subtracted. Mass-bias correction was based on external calibration using natural Cr standard solution and IUPAC isotopic abundances. This is a standard bracketing technique where the measured isotope abundances are compared with the certified values of a standard.[9] The following ion intensities were

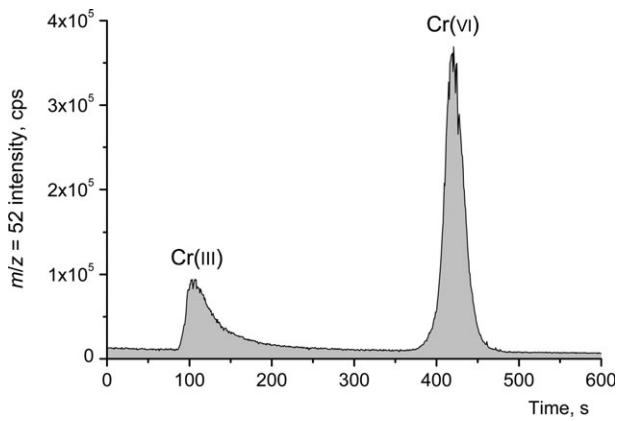

Fig. 1 Example chromatogram of Cr(III) and Cr(VI) determination from yeast extract using isotope dilution HPLC-ICP-MS (^{52}Cr trace shown).

Table 1 Comparison of the Cr determination results

	Iterative solution (USEPA)[a]	Non-iterative solution[b]	Isotope pattern deconvolution
α	0.2232	0.2232	0.2232
β	0.3818	0.3818	0.3818
$n_{Cr(III)}$/μmol	7.3672	7.3672	7.3672
$n_{Cr(VI)}$/μmol	0.1718	0.1718	0.1718

[a] After 6 iterations. [b] Solved analytically using MathCad v.12.

obtained after the mass-bias correction:

$$\begin{array}{c} \quad\text{Cr(III)}\quad\text{Cr(VI)} \\ \begin{matrix}50\\52\\53\end{matrix}\begin{pmatrix}3\,301\,380 & 3\,075\,887\\ 2\,953\,840 & 3\,242\,343\\ 3\,263\,570 & 15\,684\,642\end{pmatrix} \\ \textit{before mass-bias correction}\end{array} \mapsto \begin{array}{c} \quad\text{Cr(III)}\quad\text{Cr(VI)} \\ \begin{matrix}50\\52\\53\end{matrix}\begin{pmatrix}3\,359\,763 & 3\,130\,282\\ 2\,953\,840 & 3\,242\,343\\ 3\,220\,475 & 15\,477\,528\end{pmatrix} \\ \textit{after mass-bias correction}\end{array}$$

These ion intensities were subjected to eqns (7) and (8) and the multiple regression analysis was performed using the Microsoft Excel® LINEST array function (regression intercept is set to zero). Note that there is no need to calculate the isotopic abundances of the analysed samples since mass-bias corrected ion intensities are directly used in eqns (7) and (8). For example, to obtain a_1, b_1 and c_1, the peak areas from the chromatographic peak of Cr(III) are used as the y-values (a 3 × 1 matrix) and the isotopic abundance 3 × 3 matrix is the known x-values of the multiple regression. The following estimates of the slopes were obtained from both peaks of Cr(III) and Cr(VI):

$$\begin{pmatrix}a_1 & b_1 & c_1\\ a_2 & b_2 & c_2\end{pmatrix}=\begin{pmatrix}3\,306\,810 & 2\,988\,815 & 3\,322\,029\\ 3\,068\,957 & 15\,635\,569 & 3\,245\,020\end{pmatrix}$$

The accuracy of the obtained slope values can always be verified using the back-propagation method. For example, the m/z 52 ion intensity in the Cr(III) peak must equal $a_1 \cdot A^{50}_{Cr(III)s} + b_1 \cdot A^{50}_{Cr(VI)s} + c_1 \cdot A^{50}_{Cr}$, *i.e.*, 3 306 810 × 0.9720 + 2 988 815 × 0.0004 + 3 322 029 × 0.0435 = 3 359 923 (compare with the measured mass-bias corrected value of 3 359 763). Once the regression slopes are established, the values of α and β are obtained using eqn (10) and the amounts of Cr(III) and Cr(VI) in the analysed yeast sample (μmol) are calculated from eqn (12).

The ability to make a simple estimate of α and β is also very important in method development since these variables are a quantitative measurement of the quality of a particular speciation method. Eventually, the method with the lowest α and β values is the most preferable in speciation analysis (USEPA recommends that $\alpha + \beta < 0.8$).[6] Species interconversion values can also be used in metabolism studies. For example, isotope pattern deconvolution was recently applied to calculate the amount of mono-, di- and tributyltin interconversion (debutylation) in rats.[11]

It is important to stress that the numerical values from the isotope pattern deconvolution approach are in exact agreement with the USEPA iterative two-isotope ratio approach and with the analytical (non-iterative) solution of the same fundamental isotope dilution equations (see Table 1).

The above approach can also be directly applied to Hg(II) and $MeHg^+$ determination using double spiking to determine the extent of Hg(II) methylation and $MeHg^+$ demethylation during sample analysis.[12] This approach can also easily be extended to triple spiking experiments. Similar to the double spiking example, the simplicity of the isotope pattern deconvolution equations is in sharp contrast to the complexity of the conventional two-isotope ratio approach.[13]

Conclusions

We have shown that complex calculations of double spike ID results with species inter-conversion based on the conventional two-isotope ratios can be simplified when least-squares ion intensity-based isotope pattern deconvolution is used instead. The complexity in two-isotope-ratio calculations arises from the fact that all the two-isotope ratio equations must be stitched together to obtain the unknown parameters, while the isotope pattern approach handles the same calculation in one step. As a result, the isotope pattern deconvolution approach delivers a more intuitive and elegant solution to otherwise complex data analysis without the need of iterative calculations as is widely practised in double spiking isotope dilution.[4–6]

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